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Attorney Docket No. TMT-10902/04

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael T. Trese et al.

Serial No.: 10/068,314 Group Art Unit: 3763

Filed: February 6, 2002 Examiner: Matthew F. DeSanto

For: METHOD FOR VITREOUS LIQUEFACTION

DECLARATION OF PATRICK J. GAFFNEY

I, Patrick J. Gaffney, hereby declare as follows:

1. I am a citizen of the Irish Republic and a resident of Great Britain presently residing at 27 Milton Road, Harpenden, Hertfordshire, United Kingdom.

2. I am a former Visiting Professor in the Department of Medicine at the University of Leuven, Belgium and in the Department of Human Anatomy and Physiology at University College, Dublin, Ireland. I am currently an Honorary Senior Research lecturer in the Academic Department of Surgery at St. Thomas's Hospital, London, United Kingdom.

3. My academic background and experience in the field of the present invention are listed in the attached curriculum vitae.

4. I have read the reference Trese et al. that I understand is being construed by the United States Patent and Trademark Office as using plasmin to cause vitreal liquefaction.

5. By way of this declaration, I intend to demonstrate that Trese et al., while reporting to the contrary, actually: (a) created a preparation containing a high percentage of streptokinase-plasminogen complex and only a small amount of free plasmin; (b) streptokinase-plasminogen complex has very little proteolytic activity; and (c) that the

inconsistent liquefaction recited in Trese et al. is therefore attributable to the predominant species in their preparation being this streptokinase-plasminogen complex.

6. Trese et al. created a preparation containing primarily the streptokinase-plasminogen complex and only a small amount of plasmin. In the original paper of Trese et al. (1), the authors have described an approximate 1:1 molar ratio mixture of streptokinase (SK) and plasminogen (Plgn) as an adjunct in vitreous surgery. It is known that such a mixture gives rise to a reasonably stable SK-Plgn complex, which has the ability to activate plasminogen to plasmin (2,3). It would seem that this equimolar complex has an active site exposed, which allows interaction with small chromogenic peptides (e.g., S-2251 or Chromozym – PL) such that the assay of this complex is the most common and effective method to assay intact plasminogen in plasma (4). The interaction of SK with Plgn is quite complex and has been reviewed. The review summarizes the composition and behavior of the various complexes derived from the combinations of SK and Plgn (5). Streptokinase does not form an activator complex with all vertebrate plasminogens and has a specificity for human, primate, cat and other species of plasminogen, while not reacting with bovine and some other plasminogens (6,7).

7. The SK-Plgn complex has very little proteolytic activity, while it can degrade casein slowly. The major biological purpose of the SK-Plgn complex is to activate the proenzyme plasminogen to active plasmin.

8. There is a consensus that effective plasminogen to plasmin conversion with SK is achieved by generating a small amount of activator by adding a small quantity (less than 10%) of SK to the plasminogen solution. The resultant small quantity of the SK-Plgn will rapidly activate the remaining plasminogen to plasmin. The procedure for obtaining

plasmin in the current patent application involves mixing approximately 11.3 nmoles of plasminogen derived from 22 mls of autologous plasma with 1.3 nmoles of SK. This should (as described above) yield 1.3 nmoles of the SK-Plasminogen (SK-Plgn) activator complex and this, in turn, will rapidly convert the residual 10 nmoles of plasminogen to active plasmin. This procedure is expected to give a more hydrolytically active preparation with a greater possibility of consistency from preparation to preparation. Conditions of storage prior to use should be such (e.g. 4°C or below) as to reduce the autodegradative process, which would reduce the potency of a preparation. This degradation can be monitored by electrophoretic assessment of the hydrolytic cleavage of the active site chain by plasmin (8).

9. The following references are cited as part of this declaration:

(1) Trese, MT, Williams, GA, Hartzer, MK (2000) Ophthalmology; 107, 1607-1611.

(2) Robbins, KC, Summaria, L, Hsieh, B, Shah, RJ. (1967) J Biol Chem; 242, 2333-2342.

(3) Reddy, KNN, Markus G. (1974) J Biol Chem; 249, 4851-4857.

(4) Gaffney, PJ (1998) In: Laboratory Techniques in Thrombosis – a Manual. (eds: J Jespersen, RM Bertina, F Haverkate) Kluwer Academic Publishers, Netherlands, pp 247-255.

(5) Robbins, KC, Markus, G. (1977) In Fibrinolysis – Current Fundamental and Clinical Concepts (Eds. PJ Gaffney, S. Barkuv-Ulutin) Academic Press, London, pp 61-75.

(6) Wulf, RJ, Mertz, ET. (1969) Can J Biochem; 47, 927-931.

(7) Siefring, GE, Castellino, FJ. (1976) J Biol Chem; 251, 3913-3920.

(8) Gaffney, PJ, Brasher, M, Lord, K, Kirkwood, TBL (1977) Haemostasis; 6, 72- 88.

10. Thus, to summarize, the recitation in Trese et al. as to plasmin enzyme being the active species injected into eyes is simply incorrect. According to the method by which Trese et al. indicates on page 1608 for "Preparation of Autologous Plasmin Enzyme," I am

convinced that the resulting material was not in fact plasmin but instead a streptokinase-plasminogen complex as I have described above. Since it is my understanding that this reference has been cited in the course of the patent process for disclosing plasmin injection at 0.4 units, it is my assertion that following the procedure of Trese et al. the vast majority of the 0.4 units injected was in fact streptokinase-plasminogen complex. Thus, Trese et al. does not in fact show the use of 0.4 units of plasmin as a therapeutic reagent to induce PVD.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Patrick J. Gaffney

Date: Oct 30, 2003

Attorney Docket No. TMT-10902/04

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Michael T. Trese et al.

Serial No.: 10/068,314 Group Art Unit: 3763

Filed: February 6, 2002 Examiner: Matthew F. DeSanto

For: METHOD FOR VITREOUS LIQUEFACTION

SUPPLEMENTAL DECLARATION OF MICHAEL K. HARTZER, Ph.D.

I, Michael K. Hartzer, hereby declare as follows:

1. I reaffirm my statements made in my previous declaration of May 9, 2003.

2. Plasmin (PL) is a hydrolytic enzyme that can degrade a variety of proteins in blood and in the vessel wall. An earlier name had been fibrinolysin, which reflects the major activity of plasmin (e.g. the lysis of fibrin). Essentially there is little or no free plasmin in blood, while its enzymatically-inactive proenzyme form, plasminogen (plgn), is present at 2 μM in human plasma. While plasminogen in plasma may be measured by both indirect and direct methods, plasmin activity can only be measured by a direct method. This is reviewed elsewhere (Gaffney PJ, et al. (1977) Haemostasis 6: 72-881). Indirect methods to measure plasminogen involve the conversion of inactive plasminogen to the active plasmin using a variety of well-known activators, notably Streptokinase (SK), Urokinase (UK) and Tissue-type plasminogen activator (t-PA). The resultant active plasmin can be measured using synthetic substrates or natural biological substrates. A number of synthetic substrates, which mimic the natural protein substrate, have been developed in order to make the assay simple and practical. Typically, a chromogenic group is attached to a plasmin-specific peptide with an amide bond. The most commonly used synthetic substrate for plasmin is S-2251 (D-Val-

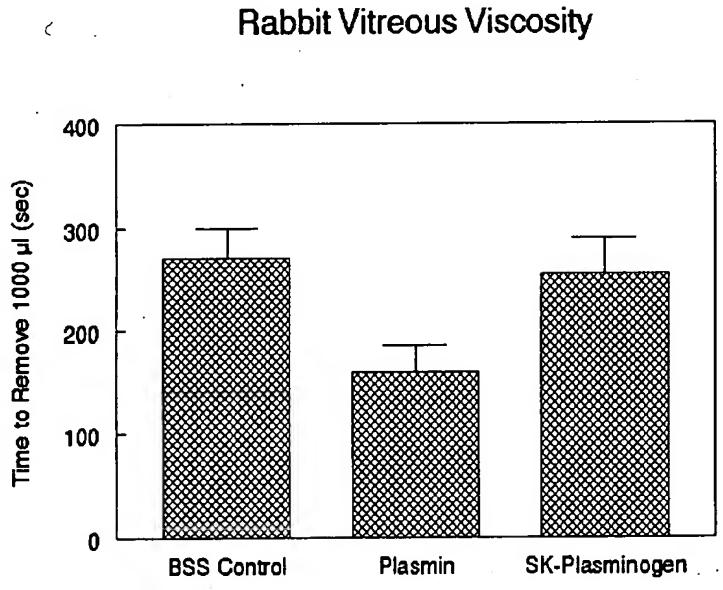
Leu-Lys-p-Nitroanilide Dihydrochloride). When the carboxy-terminal peptide bond is cleaved, the p-Nitroaniline is released from the peptide and is measured as a yellow color at a 405 nm wavelength. The hydrolysis of the S-2251 by plasmin follows a Lineweaver-Burke kinetic relationship.. This means that if the substrate (S-2251) is present at a sufficiently high concentration, the amount of color at any one time is dependent upon the amount of enzyme used in the reaction.

3. SK-plasminogen complex levels in plasmin preparation used in Trese et al. (Ophthalmology 2000; 107:1607-11) were prepared as follows: 30 mls of blood were drawn in this study. This yields approximately 15 mls of plasma. Plasminogen levels were quantified in preparations prepared according to the method described in Trese et al. In 10 preparations, an average of 0.75 ± 0.14 nmoles of plasminogen were recovered per ml of plasma. Therefore, in a preparation starting with 15 mls of plasma, the average yield would be 11.3 nmoles of plasminogen. In the method described in Trese et al., 50,000 IU of streptokinase was added to the plasminogen. According to the manufacturer, this represents 10 nmoles of streptokinase. Since streptokinase rapidly forms a 1:1 complex with plasminogen, an average of 88% of the plasminogen would be present as the complex. The other 12% of the plasminogen is converted to plasmin.

4. Subsequent to the publication of Trese et al., I developed strong experimental evidence that plasmin has much higher biological activity than the streptokinase-plasminogen complex. My experimental evidence was confirmed in discussions with Professor Patrick Gaffney, an expert in the field of plasmin fibrinolysis. We believe that the limited proteolytic activity seen in our plasminogen preparations activated with approximately equimolar amounts of streptokinase (Trese et al) was due to the small amount of free plasmin in the preparation. One possible reason for low proteolytic activity of the streptokinase-

plasminogen complex is that it is a much larger molecule than plasmin. Since proteins have three-dimensional structure, it is common that cleavage sites on substrate proteins (e.g. fibrinogen, laminin and fibronectin) are not on the surface of the substrate protein but rather located within an internal crevice, pocket or fold. It is hypothesized that the increased size of the streptokinase-plasminogen complex limits its ability to access such cleavage sites on a substrate protein. As a result of the increase in biological activity for plasmin, the variability in liquefaction of vitreous gel reported in Trese et al. at doses of 0.4 units or less is dramatically reduced when plasmin and not streptokinase-plasminogen complex is the active enzyme.

5. The experimental support for the increased biological activity of plasmin relative to streptokinase-plasminogen complex is shown in the following graph that measures rabbit vitreous viscosity.



BSS, Plasmin (1.0 U) and SK-Plasminogen (1.0 U) were injected into rabbit eyes (n=5). The activity of the injected enzyme preparations was assayed using a synthetic substrate (S-2251). Vitreous liquefaction was assayed 24 hours later by measuring the time required to remove

1000 µl of vitreous with a 25 ga vitrectomy instrument using a standardized procedure for removal. The time required to remove the plasmin vitreous (161 ± 24 sec) was significantly lower than the time required to remove the vitreous in the other groups ($p < 0.05$). This study provides evidence that plasmin liquefies rabbit vitreous whereas SK-plasminogen complex does not.

6. As a result of the steps taken in the present invention to induce the formation of free plasmin that has higher biological activity than the streptokinase-plasminogen complex, it is possible according to the present invention to consistently perform liquefaction of the vitreous gel at plasmin doses of 0.4 units or less. I believe the pending claims vary from the teaching of Trese et al. in that Trese et al. had in actuality as an active enzyme a streptokinase-plasminogen complex and not free plasmin.

7. Based on the above considerations, I do not believe that Trese et al. teaches vitreous liquefaction in a human eye at a plasmin dose of less than 0.4 units.

8. As part of this supplemental declaration, it is intended to establish completion of the invention being claimed in the above-referenced application within the United States at a date prior to December 5, 2001, which is the effective date of X. Lei, X. Shi, and J. Fan, "Posterior vitreous detachment with plasmin in the isolated human eye," Graefe's Arch Clin Exp Opthalmol (2002) 240:56-62 which was cited in the non-final Office Action mailed December 20, 2002.

9. The months leading to our reduction to practice were devoted to experimentation. In support of this statement, appended to this declaration is a page from my laboratory showing some early experimental results indicating that the amount of plasmin, as opposed to a streptokinase-plasminogen complex, varied as a function of preparation technique. This page shows invention prior to the publication of Li et al.

10. The disclosure corresponding to the above-referenced application was forwarded to the law firm of Gifford, Krass, Groh, Sprinkle, Anderson & Citkowski on November 14, 2001. This disclosure resulted in the filing of the above-referenced patent application on February 6, 2002. Appended hereto is a docket page from our attorneys confirming this date.

11. Based on the above considerations, I do not believe that the Li et al. reference published online December 5, 2001 preceded my actual reduction to practice of the invention in this application.

12. This declaration is submitted simultaneous with a request for continued examination and in response to rejections relying on Li et al. in the final Office Action mailed July 30, 2003.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Michael K. Hartzer, Ph.D.

Date: 10/17/03

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